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Breitling, Rainer

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Opinion

Robust signaling networks of the adipose secretome

Rainer Breitling

Groningen Bioinformatics Centre, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Type 2 diabetes is a prototypical complex systems disease that has a strong hereditary component and etiologic links with a sedentary lifestyle, overeating and obesity. Adipose tissue has been shown to be a central driver of type 2 diabetes progression, establishing and maintaining a chronic state of low-level inflammation. The number and diversity of identified endocrine factors from adipose tissue (adipokines) is growing rapidly. Here, I argue that a systems biology approach to understanding the robust multi-level signaling networks established by the adipose secretome will be crucial for developing efficient type 2 diabetes treatment. Recent advances in whole-genome association studies, global molecular profiling and quantitative modeling are currently fueling the emergence of this novel research strategy.

The diabetes pandemic

Populations worldwide are currently experiencing an epidemic of obesity with many associated health problems, including type 2 (non-insulin-dependent) diabetes, atherosclerosis and certain cancers [1]. Type 2 diabetes is a complex, heterogeneous, polygenic disease that affects more than 150 million people worldwide, and large increases in these numbers, particularly in developing countries and among adolescents, are predicted for the coming years [1,2]. The consequences of the disease can be devastating, ranging from cardiovascular morbidity to microvascular complications that result in, for example, blindness and lower limb amputation.

Type 2 diabetes is the result of a long-term systemic disturbance involving many organ systems, rendering this disease particularly attractive for using a quantitative systems biology approach [3]. This is particularly the case for the complex regulatory networks established by secreted molecules from adipose tissue during the development of diabetes.

Diabetes as a multi-level, system-wide disease

Diabetes ultimately is a disease of the pancreas. Pancreatic β-cells, the insulin-producing cells, have a central role in adaptive responses to compensate for insulin resistance (see Glossary). During development of diabetes, β-cells are overloaded by the increased demand for insulin secretion and begin to undergo apoptosis [1]. However, many other tissues contribute to the gradual development of global insulin resistance that precedes overt diabetes. Together with the liver [4], brain [5] and skeletal muscle [6], the adipose tissue is a central player in regulating diabetes progression. This role is mainly determined by the adipose secretome, the huge diversity of soluble signaling factors that are secreted from adipose tissue that render it a major endocrine organ [7–9]. In addition, ectopic fat in muscle (intramyocellular lipids) and liver has been suggested to contribute to both insulin resistance and chronic inflammation [10], which, as will be discussed later, is a major pathogenic factor in diabetes progression.

The complexity of the adipose tissue secretome

The realization that adipose tissue acts as an endocrine gland affecting whole-body energy homeostasis was a major breakthrough towards a better molecular understanding of type 2 diabetes [7,8]. Secreted factors from adipose tissue (adipokines) form a complex network of cell-to-cell and organ-to-organ signals. Positive feedback loops in that network can turn into molecular 'vicious circles' and lock the diabetic system in an almost irreversible diabetic disease state [11].

The first adipose-tissue-derived hormone discovered was leptin, a peptide hormone that can be considered a direct indicator of the size of body fat pools [12]. It acts via cognate receptors in the brain to orchestrate the suppression of appetite and stimulation of energy expenditure in

Glossary

Adipokines: also known as adipocytokines. Cell-to-cell signaling proteins and peptides secreted by various cells in the adipose tissue, including adipocytes and tissue macrophages. Adipokines act locally or systemically by modulating the function of the immune system. More than a dozen major adipokines are currently known.

Adipose secretome: the entirety of signaling molecules secreted by adipose tissue including hormones, adipokines and lipids.

Insulin resistance: the absence of an adequate response to normal levels of insulin, which often precedes the development of diabetes.

Parameter information: quantitative information about molecular concentrations and reaction kinetics, which is required for detailed predictive modeling of signaling pathways.

PPARγ: peroxisome proliferator-activated receptor γ. Nuclear receptor with major effects on adipocyte differentiation. PPARγ is the main target of antidiabetic drugs of the thiazolidinedione type.

Robustness: the ability of a biological system to function in a variety of environmental conditions. In advanced diabetes, the diseased system exhibits robust properties that make it unresponsive to medical intervention.

Semi-quantitative hybrid models: computational models of biological systems that combine qualitative (topological) descriptions of molecule–molecule interactions and quantitative descriptions of individual reactions. Such models can be particularly useful in the absence of comprehensive parameter information.

TNFα: tumor necrosis factor α. Cytokine involved in chronic inflammation. Secretion of large amounts of TNFα as an adipokine is supposed to have a major role in the maintenance of a chronic inflamed state in diabetes.

Corresponding author: Breitling, R. (r.breitling@rug.nl).

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times of excess food supply. The absence of leptin results in uncontrolled hyperphagia-induced obesity and associated diabetes. However, most obese diabetic individuals do not have decreased leptin levels. Tumor necrosis factor α (TNFα), a second notable component of the adipose secretome, was identified as such because obese-insulin-resistant adipose tissue produced elevated amounts of this protein [13]. This surprising finding indicated that an inflammatory process contributes to type 2 diabetes progression, a concept that has become central to understanding the disease process. Since the initial groundbreaking discoveries of leptin and TNFα, the known adipose secretome has grown rapidly in diversity and now includes more than a dozen major molecules [14,15], with new players continuously being discovered, including novel adipokines, peptide hormones and endocrine-active fatty acid derivatives [16].

For a successful systems biology approach, it is necessary to take into account the regional differentiation of adipose tissue. Most importantly, visceral adipose tissue, which constitutes only a small fraction of all body fat (on average, 18%; subcutaneous fat stores comprise approximately 82% [17]), has a special part to play and is most closely related to diabetes development. By releasing free fatty acids directly into the portal circulation, visceral adipose tissue exerts a particularly strong effect on the liver, interfering with insulin signaling, but it also has a specific secretome composition, with some adipokines preferentially produced here [18,19].

I argue that the adipokine network, which comprises the peptide and lipid secretome of adipose tissue (including infiltrated macrophages) and their major target tissues, is the major contributor to the system behavior of energy control and should be the target of a concerted quantitative systems biology approach [3,11]. The properties of this network are likely to underlie many of the robust characteristics leading to treatment-resistant metabolic syndrome (Figure 1), mainly for two reasons: first, the multitude of adipokines constitutes a highly redundant system, in which different adipokine signals can act as back-up for each other, so the entire system will be robust against the loss of any individual component (including the inhibition of single molecules by anti-diabetic drugs). Second, as Figure 1 shows, the pleiotropic action of virtually all adipokines creates a tightly interconnected web of feedback loops that cannot be pulled apart into linear subsystems. Each positive feedback loop by itself constitutes a potential fragility of the system because it can turn into a self-reinforcing 'vicious circle' once the containing power of the major negative feedback loops is overwhelmed – for example, by persistent oversupply of energy (Box 1). Mono-causal explanation, just like single-target interventions, will not do justice to such a complex system.

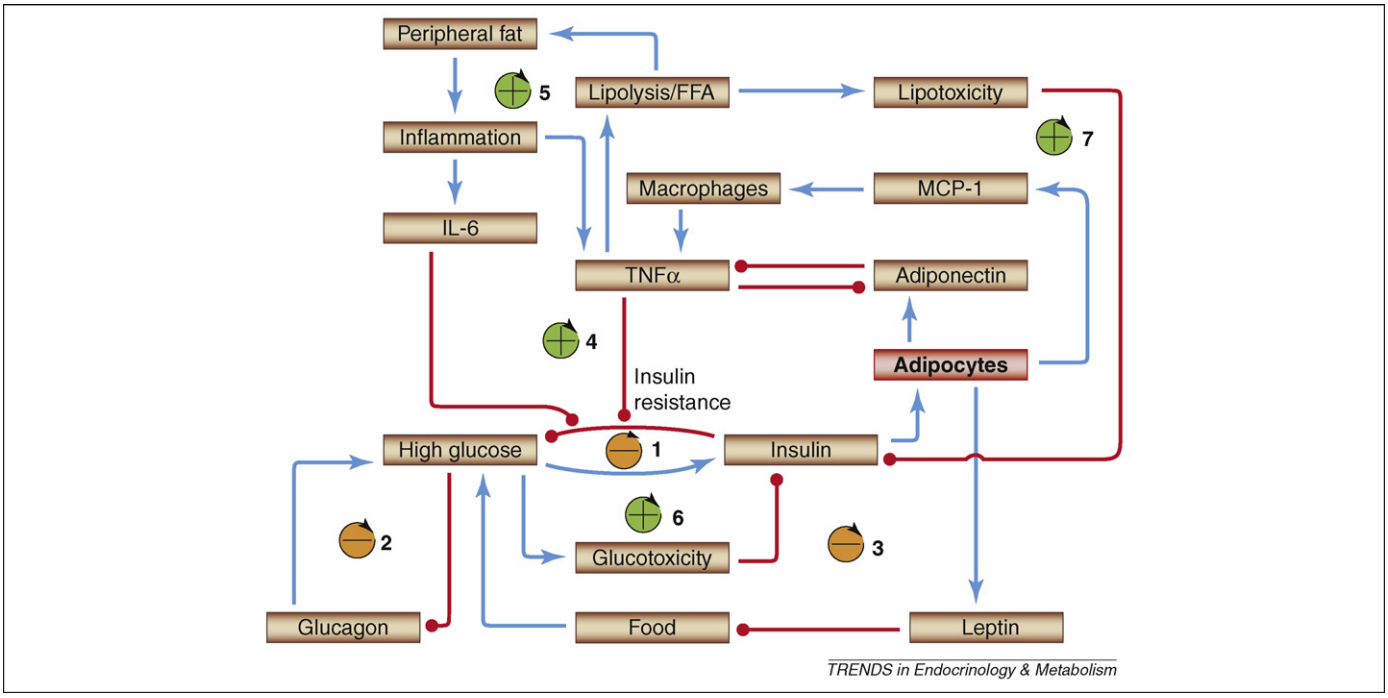

Figure 1. Sketch of major system-wide feedback loops mediated by the adipose secretome. Three negative feedback regulatory mechanisms work together to maintain serum glucose levels within narrow limits. Loop 1: high glucose levels lead to release of insulin from β cells in the pancreas, which results in glucose uptake in peripheral 'sinks', including adipocytes. As a result, glucose levels return to normal. Loop 2: low glucose levels stimulate glucagon release, which increases glucose levels via gluconeogenesis and release of alternative fuels. Loop 3: increased adipose tissue volume leads to increased leptin levels, repressing food intake and increasing energy expenditure. In conditions of continuous oversupply of energy, multiple positive feedback loops take over the system, which contribute to establishing a vicious circle that is hard to break by pharmacological treatment. Loop 4: chronic inflammation is maintained by the release of pro-inflammatory cytokines (IL-6 and TNFα), both peripherally and by tissue macrophages recruited to obese adipose tissue by MCP-1 signaling. These adipokines in turn repress insulin action and, thus, perpetuate the state of hyperglycemia, completing the vicious circle. TNFα also increases lipolysis by adipocytes. The resulting redistribution of fat depots to peripheral organs establishes a further vicious circle (loop 5) that aggravates the spread of chronic inflammation beyond adipose tissue, as well as subsequent insulin resistance. Finally, increased glucose levels caused by insulin resistance (glucotoxicity; loop 6) and increased free fatty acids, caused by chronic inflammation (lipotoxicity; loop 7), have toxic effects on β cells and can lead to irreversible damage to the insulin secretory machinery. Additional adipokines exert major modulating influences and determine at which point the system switches towards the chronic inflammatory state. For example, adiponectin interacts in a mutually antagonistic fashion with TNFα and controls the gain of the central positive feedback loop (loop 4). (Blue arrowheads indicate positive, activating influences; red bullets indicate negative, inhibiting signals.)

Box 1. Robustness and fragility of energy homeostasis and their evolution

Low blood glucose can incur high costs in a very short time with fatal damage occurring within minutes, whereas the adverse consequences of high glucose accumulate slowly over a prolonged period of many years (acute, catastrophic versus chronic, gradual effects) [11]. The robustness $R(s)$ of the energy control system (s) is the integral of the product of the probability $p(E)$ of experiencing a particular environmental challenge (E) and the associated performance gain g(E) [20],

$$R(s) = \int_E p(E)g(E)dE. \qquad \text{(Equation I)}$$

In most situations, $p(E)$ can be considered constant, so evolution has to optimize the system in such a way that the loss of performance incurred by frequent perturbations is minimized. This process is illustrated in Figure I. Historically, energy supply was close to starvation levels for prolonged periods and low glucose supply was a frequent threat, so the system contains redundant mechanisms to protect the body against hypoglycemia. Close to the 'glucose floor', the minimum viable glucose level [11], strict purifying selection will maintain a fine-tuned redundant control system. At the other extreme, the 'glucose ceiling' (the maximum tolerable glucose level), selection is much more relaxed and polymorphisms can accumulate without much negative consequence. This historical probability distribution is shown in Figure Ia.

However, as Kitano [11] argues, this causes the danger that the robust system will undergo catastrophic failure if the environment changes in an unexpected fashion. This seems to be happening on a large scale during the recent pandemic of obesity-associated diabetes (Figure Ib). Recent changes in lifestyle and diet have lead to a dramatic shift in the function $p(E)$. Starvation is no longer a threat in many societies, but oversupply of calories is almost ubiquitous. Consequently, what was once a robust system is revealed suddenly as being dangerously fragile.

The general validity of this systems biology version of the 'thrifty genotype' hypothesis is illustrated by the fact that there are several single-gene mutations in anorexigenic, appetite-controlling signals that lead to system failure (obesity). By contrast, there are hardly any cases of single-gene mutations that break the orexigenic, appetite-stimulating system. The mechanisms assuring sufficient accumulation of energy are apparently designed in a much more robust fashion than those that limit energy intake. The mechanisms underlying such a robust behavior can be the same as in engineered systems: redundancy, feedback control, modularity and structural stability [20]. The latter two are widely found in biological systems, whereas the first two are strikingly exemplified by the adipose secretome signaling network.

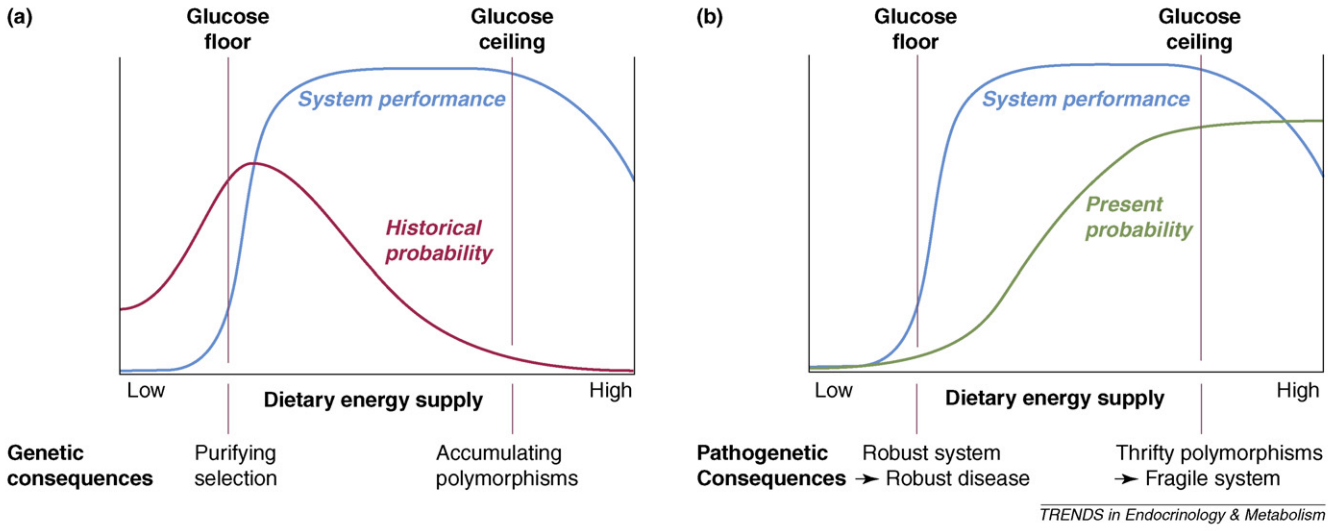

Figure I. The selection landscape shaping the energy-control system. The blue curves show the performance of the system as a function of energy supply, with rapid loss of function close to the glucose floor and gradual deterioration close to the glucose ceiling. The red line in **(a)** shows the historical probability of different energy supply levels, with highest probability close to starvation level. The green line in **(b)** shows the present situation, in which there is a high probability of energy oversupply.

Understanding diabetes progression by systems biology

The most important systems biological property of energy metabolism is the 'robust' phenotype of the diabetic patient [11,20] (Box 1): once a certain physiological threshold has been passed, the disease process becomes virtually irreversible, inducing severe side-effects for the patient (Figure 1). By contrast, at early stages of the disease, simple caloric restriction and exercise can stop progression of and even reverse insulin resistance. It is during these early stages that drug interventions might turn out to be most effective.

Quantitative modeling will be essential for the development of more effective therapies to target the later phases of the disease, during which the insulin-resistant state is locked in by several vicious positive feedback loops (Figure 1). Currently, many drugs are aimed at single targets, but some of the most effective drugs for diabetes are those that have a multitude of pleiotropic effects – for example, the thiazolidinediones, which not only stimulate a master regulator of adipocyte differentiation (peroxisome proliferator-activated receptor γ, or PPARγ) but also modulate insulin sensitivity, lipolysis and the composition of the adipose secretome [9]. Unfortunately, thiazolidinediones are now under scrutiny for potentially increasing the cardiovascular death rate [21]. Targeting multiple breakpoints of the diseased system at the same time, while still finding the right balance between intended and adverse effects, will require a large-scale quantitative modeling approach.

Chronic inflammation underlying type 2 diabetes progression

The importance of a system biology approach to type 2 diabetes is perhaps most strikingly illustrated by the molecular signaling networks that link obesity, adipokine

secretion, chronic inflammation and the development of insulin resistance [22–27]. One of the most important effects of adipokine secretion might be the recruitment of immune cells to adipose tissue in obesity, where they are activated to become the major source of further adipokine production, perpetuating a state of chronic inflammation. This is not the classic version of inflammation, characterized by calor (heat), rubor (redness), dolor (pain) and tumor (swelling), but shares many of the same cellular processes. The adipose secretome has a central role in the establishment and robust maintenance of this chronic inflamed state. The molecular mechanism involves multiple points of cross-talk between insulin and inflammatory signaling (Figure 1). Several inflammatory mediators released from macrophages in the obese adipose tissue, in particular TNFα and interleukin-6, suppress insulin signaling in adipocytes.

Technology drives discoveries

Molecular biology and clinical chemistry have led to a detailed picture of diabetes progression. It is clear that the adipose secretome contributes to a complex circuit of interwoven feedback loops that are still only partly resolved. The detailed quantitative effects of the adipose secretome on its target tissues, in addition to its synergistic and antagonistic actions, still remain to be determined. New technologies and concepts will be instrumental in deciphering the pertinent molecular networks and their quantitative relationships.

Three complementary novel approaches have been added recently to the toolbox of technologies for exploring the systems biology of diabetes on a large scale: first, whole-genome association studies identify additional components of the system; second, new molecular profiling techniques determine their functional interaction in cellular networks; and finally, predictive computational models combine this information to explore the non-linear disease dynamics that emerge in these molecular networks. These three approaches are discussed below.

Genetic approaches towards system reconstruction

Type 2 diabetes is a disease with a multifactorial etiology. It is strongly influenced by environmental factors, most importantly food intake and lifestyle, but it also has a strong genetic component. In fact, offspring of an affected individual show a lifetime risk of 38% of developing the disease, approximately two to three times higher than average; furthermore, siblings of a diabetic patient are two to three times more likely to develop the disease than others [1]. Moreover, the concordance rate in monozygotic twins is approximately twice as high as in dizygotic twins (76% versus 40% [28]). The mechanism underlying the inherited differences in susceptibility is complex, making diabetes a 'geneticist's nightmare' and a prototypical complex disease [29].

Targeted attempts at finding causative polymorphisms among candidate genes such as TNFα or insulin receptor substrate-1 (IRS1) were very difficult to replicate in subsequent studies [1,29]. In fact, polymorphisms in many genes were shown to contribute relatively small effects. The most robust candidate gene polymorphism associated with diabetes affected PPARγ, a crucial regulator of adipocyte differentiation and physiology and, also, the major target of anti-diabetic drugs of the thiazolidinedione family [9]. Carrying the low-risk allele for this gene leads to a risk reduction of ~20%.

Recently, whole-genome association studies have led to an explosion in our knowledge of genetic modifiers of diabetes susceptibility [28,29]. Within a single year (2007), the number of strong, well-replicated candidates has more than doubled. This has revealed some general patterns: on the one hand, a large number of the newly confirmed diabetes-associated genome loci contain known diabetes-associated genes that act in the pancreas to modulate β-cell insulin secretion and/or adaptive growth. Frayling [29] provides a detailed list and further discussion. On the other hand, many other genes that have been identified with reproducible effects would not have been considered obvious candidate genes. For example, the high-risk allele of *TCF7L2*, a widely expressed transcription factor, confers a higher diabetes risk than any candidate gene examined before – but before its detection in the large-scale association studies, the gene had only been studied as a transcriptional regulator involved in Wnt signaling during development and cancer [30,31].

It is important to realize that the genetic polymorphisms identified in these genome-wide studies are not predicted to lead to a functional null allele. Rather, it is expected that relatively subtle differences in activity cascade through the system and lead to ultimate failure of the insulin signaling system. In addition, common 'disease' alleles will be present in diverse combinations in individual persons; each patient will have his or her unique molecular mechanism leading to the disease. Computational models that describe the non-linear, dynamic interactions between all molecular players involved will be crucial for understanding how various polymorphic loci contribute to the disease in genetically diverse individuals [32].

Comprehensive molecular profiling

The full realization of the complexity of the adipose secretome was, to a large extent, the result of the post-genomic technology revolution. For instance, adiponectin, the major 'beneficial' adipokine (which at high concentrations improves insulin sensitivity), was initially discovered as *apM1* (adipose most abundant gene transcript 1), an abundantly expressed adipocyte gene, in untargeted studies of the transcriptome using gene-expression microarrays [33]. Several other adipokines, like chemerin [14] and visfatin [34], were discovered in a similar way. Recently, comprehensive transcriptome studies have measured gene expression in large cohorts of genetically characterized individuals, including healthy and diabetic persons. By determining which genomic loci influence gene-expression levels, these studies have created global overviews of the gene-regulatory networks that are active in obesity and diabetes [35–37].

To get a more intimate insight into the relevant molecular mechanisms of diabetes development, it will be necessary to extend these studies beyond gene expression. One particularly promising approach towards understand-

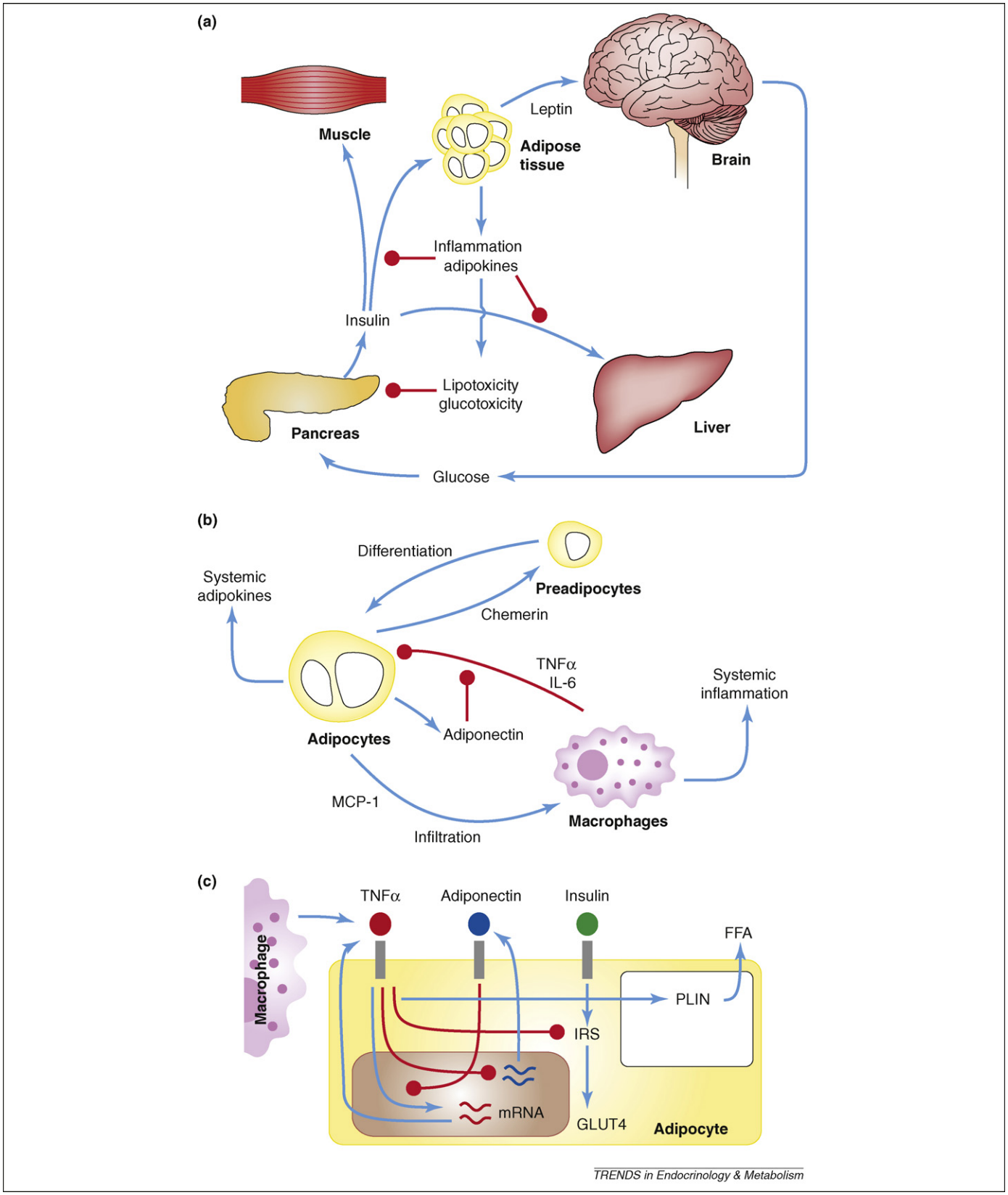

Figure 2. Multiple scales of regulatory circuits in energy homeostasis. The adipose secretome contributes to feedback loops that operate at multiple levels in the diabetic system. Computational models need to cover all these scales in an integrated description. The figure illustrates the overall structure of the networks; for additional details, see the literature cited. **(a)** Inter-organ networks. In development of diabetes, chronic inflammation of adipose tissue leads to production of free fatty acids and inflammatory adipokines, ultimately leading to generalized insulin resistance. This results in persistently increased levels of glucose and disrupts insulin production and release in the pancreas, leading to further disease progression [1,56]. **(b)** Within-tissue networks. Obese adipocytes trigger infiltration of macrophages into adipose tissue. The resulting chronic inflammation is mainly mediated by adipokines such as TNFα and interleukin. Resulting changes in the adipose tissue secretome spread the effect to peripheral organs and also cause autocrine effects on adipocyte differentiation [14]. **(c)** Intracellular networks. Complex intracellular signaling networks regulate adipokine secretion in response to the current status of the adipose secretome [2,57]. (Blue arrowheads indicate positive, activating influences; red bullets indicate negative, inhibiting signals.)

ing adipose secretome dynamics is proteomics, the comprehensive profiling of proteins in a sample [38,39]. This can be applied to quantitatively determine post-translational modifications and aggregation states of secretome components such as adiponectin (which occurs in several multimeric forms with distinct action profiles) in both cell culture and tissue explants. It is also now possible to measure the kinetics of intracellular signaling cascades in a comprehensive fashion, using quantitative proteomics to selectively measure the phosphorylated proteins that turn adipokine signals into cellular responses [40].

Metabolomics, the molecular profiling of small molecules [41,42] and, in particular, lipidomics, which focuses on lipids and their derivatives [43,44], will also have a major role in such a comprehensive strategy. Their most important contribution will be the unbiased identification and quantification of new signaling molecules. For instance, fatty acids released from adipose tissue can be regarded as endocrine signals, and they have been shown to have diverse effects on insulin sensitivity, acting as both positive and negative modulators, depending on their site of action and molecular identity [45]. Furthermore, the differential composition of lipids stored ectopically (i.e. not in adipose tissue) could also be a key influence. Oxidized phospholipids have just recently been identified as potent modulators of the inflammatory response [46].

Quantitative modeling strategies

The complexity of the regulatory circuits underlying diabetes progression is realized on multiple scales, from whole-body models to molecular signaling cascades (Figure 2). Building system models that bridge these scales is a central challenge for diabetes research. Initial models, which describe the most important signaling processes in various relevant cell types, recently have been published and analyzed [11,32,47,48]. Such models rely on the availability of parameter values for the various molecular processes that constitute the non-linear behavior of energy metabolism and its regulation. This includes enzymatic reactions, diffusion and transport processes, signaling cascades, and even quantitative descriptions of cell proliferation and death. This type of quantitative information has been used recently to model adipose tissue metabolism and to predict physiological responses and the influence of various lipases [49]. Preferably, such models would also incorporate specific parameters for common genetic variants, which can influence the kinetics of particular enzymes or the amount of secreted signaling molecules. These values are not yet available, and the further expansion of systems biology needs quantitative input from dedicated experiments to fill this gap.

Quantitation is already widely used at the macrolevel: for instance, methods for the accurate quantification of insulin levels and insulin resistance are a common target of research, and mathematical models of diabetes physiology have a long tradition [50]. At the microlevel (from cells to molecules), quantitation is much more challenging and has been restricted to more specific aspects, such as determining individual binding affinities for receptor–ligand complexes or the activation kinetics of particular signaling pathways. Comprehensive quantification, as well as successful large-scale systems modeling, will require collaboration between many systems biology groups. Several initiatives are trying to establish the necessary standards, protocols and computational tools to make this process possible [51]. Of particular importance are common languages for describing biological models, such as the Systems Biology Markup Language (SBML) and Cell Markup Language (CellML), which greatly facilitate information exchange [52,53].

Parameter information will remain incomplete for some time. At the moment, while our understanding of energy metabolism is expanding rapidly and new players are discovered at an astonishing rate, I predict that semi-quantitative hybrid models will be particularly powerful. They can combine qualitative reasoning ('if A increases above some threshold, B is inhibited'), parameter constraints ('the affinity of receptor R is somewhere between 10 and 500 nM'), and exact kinetic and thermodynamic information (usually expressed in differential equations describing enzymatic and transport reactions). These together can lead to concrete predictions of system behavior, even when working with incomplete knowledge [54,55].

Conclusion

The communication network established by the adipose tissue secretome is of amazing complexity. A large number of adipokine-based feedback loops interact to maintain a robust disease state that is gradually aggravating and becomes resistant to therapeutic intervention. I maintain that this multi-level network of signaling pathways is eminently suitable for a quantitative systems biology approach, which will be necessary to answer many of the remaining questions in the field (Box 2). Recent advances in the three described technological domains will, in my view, contribute to progress in this direction. Whole-genome association studies identify missing molecular players; new molecular profiling approaches quantify these players and identify their causal connections; and, finally, computational modeling approaches are used to analyze system behavior and identify critical break-points. This integrated approach will help our understanding of disease progression, its evolutionary history, and individual differences in susceptibility and pathogenesis to form the basis for a more effective, personalized treatment and prevention plan for type 2 diabetes.

Box 2. Questions for future research

- How many molecular players contributing to the regulatory complexity of the adipose secretome remain to be discovered?
- Will genome-wide association studies identify the major points of system fragility contributing to diabetes?
- How important is the secretome diversity of various subtypes of adipose tissue for the diabetic disease process?
- How can we integrate genetic, proteomic and metabolomic information into a single predictive model of adipocyte biology?
- Which computational approaches will be most efficient for a large-scale, systematic approach at modeling the adipokine regulatory network in the absence of detailed kinetic information?

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